DATA EVALUATION RECORD

GLYPHOSATE
Study Type: OCSPP 890.1200, Aromatase Assay

EPA Contract No. EP10H001452
Task Assignment No. 2-74-2012 (MRID 48671303)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
CSS-Dynamac Corporation
1910 Sedwick Road,
Building 100, Suite B
Durham, NC 27713

Primary Reviewer Joan Harlin, M.S.	Signature: Date:	Joan Harlin 7/5/2012
Secondary Reviewer Scott D. Studenberg, Ph.D., D.A.B.T.	Signature: Date:	Scott Studenberg 7/11/2012
Program Manager: Jack D. Early, M.S.	Signature: Date:	Jack D. Early 7/13/2012
Quality Assurance: Jack D. Early, M.S.	Signature: Date:	Jack D. Early 7/13/2012

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by CSS-Dynamac Corporation personnel**.**

The US EPA Endocrine Disruptor Screening Program (EDSP) Tier 1 screening battery is comprised of eleven screening assays intended to identify a chemical's likely endocrine bioactivity, i.e., its potential to interact with the estrogen, androgen, or thyroid (E, A, or T) pathways. The robustness of the Tier 1 battery is based on the strengths of each individual assay to identify potential endocrine bioactivity with complementary endpoints within the assay, where available, and redundancy across the battery. Thus, the results of each individual assay should not be considered in isolation but rather should be considered in the context of other assays in the battery as well as Other Scientifically Relevant Information (OSRI). In order to determine if a chemical has the potential to interact with the E, A or T pathways, a Weight of Evidence (WoE) evaluation of Tier 1 assay results, in combination with the findings in the OSRI, should be undertaken (refer to the WoE Document).

Primary Reviewer: Anwar Y. Dunbar, Ph.D. **Signature:** _____
Risk Assessment Branch 1, Health Effects Division (7509P) **Date:** _____
Secondary Reviewer: Greg Akerman, Ph.D. **Signature:** _____
Risk Assessment Branch 1, Health Effects Division (7509P) **Date:** _____

Template version 08/2011

DATA EVALUATION RECORD

STUDY TYPE: Aromatase (Human Recombinant); OCSPP 890.1200

PC CODE: 417300 **DP BARCODE:** D401747

TXR#: 0053233 **CAS No.:** 1071-83-6

TEST MATERIAL (PURITY): Glyphosate (95.93% glyphosate acid (85.14% calculated glyphosate content in sample)

SYNONYMS: *N*-(phosphonomethyl)glycine

CITATION: Wilga, P.C. (2012). Glyphosate: Human Recombinant Aromatase Assay. CeeTox, Inc., Kalamazoo, MI. Laboratory Study No.: 6500V-100334AROM, March 9, 2012. MRID 48671303. Unpublished.

SPONSOR: Joint Glyphosate Task Force LLC, 8325 Old Deer Trail, Raleigh, NC

TEST ORDER #: CON-417300-23

EXECUTIVE SUMMARY: In an *in vitro* aromatase (CYP 19) assay (MRID 48671303), glyphosate (95.93% glyphosate acid; 85.14% calculated glyphosate content in sample; Lot # GLP-1103-21149-T) was incubated with human recombinant aromatase and tritiated androstenedione ([1β-^{3}H(N)]-androst-4-ene-3,17-dione; [^{3}H]ASDN) at log concentrations of 10^{-10} to 10^{-3} M for 15 minutes to assess the potential of glyphosate to inhibit aromatase activity. The solvent vehicle was 0.1 M phosphate buffer for glyphosate, ethanol for ASDN, and dimethyl sulfoxide (DMSO) for 4-OH ASDN, with a final assay volume of ≤1% DMSO.

Aromatase activity was determined by measuring the amount of tritiated water produced at the end of a 15-minute incubation for each concentration of chemical. Tritiated water was quantified using liquid scintillation counting (LSC). Four independent runs were conducted; however, the first run was not used because of incorrect standard preparation. The remaining three runs were conducted and each run included a full activity control, a background activity control, a positive control series (10^{-10} to 10^{-5} M) with a known inhibitor (4-hydroxyandrostenedione; 4-OH ASDN), and the test chemical series (10^{-10} to 10^{-3} M) with three repetitions per concentration.

Aromatase activity in the full activity controls was 0.676 ± 0.072 nmol·mg-protein^{-1}·min^{-1}. The response of each full activity control within a run was between 90 to 110% of the average full activity. Activity in the background controls ranged 0.23 to 0.38% and averaged 0.30% of the

full activity control. The response of the full activity controls and background controls was acceptable for each run.

For the positive control substance (4-OH ASDN), aromatase activity results were within the recommended ranges for the performance criteria. The estimated log IC_{50} for 4-OH ASDN averaged −7.29 M and the Hill slope was −0.96.

For glyphosate, aromatase activity averaged 0.673 ± 0.066 nmol·mg-protein^{-1}·min^{-1} at the lowest tested concentration of 10^{-10} M and 0.741 ± 0.100 nmol·mg-protein^{-1}·min^{-1} at the highest tested concentration of 10^{-3} M. The average aromatase activity was ≥99.67% of the control at all tested glyphosate concentrations for all runs.

Based on the data from the average response curve, glyphosate is classified as a Non-inhibitor of aromatase activity in this assay.

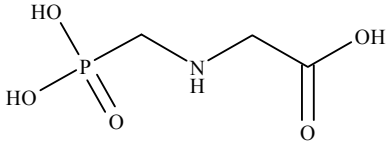
The assay **satisfies** the EDSP Tier 1 Test Order requirements for an Aromatase assay (OCSPP 890.1200).

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Substance: Glyphosate

Description:	White crystalline solid
Source:	Monsanto Company, St. Louis, MO
Lot # (expiration date):	GLP-1103-21149-T (March 9, 2012)
Purity:	95.93% glyphosate acid (85.14% calculated glyphosate content in sample)
Volatility:	Not reported
Storage conditions:	Room temperature (e.g. ambient)
Stability:	Not reported
Solvent:	0.1 M sodium phosphate buffer
Solubility (in test solvent):	10^{-3} M
Highest Concentration Tested:	10^{-3} M
Stock Solution Preparation:	Serial dilution
Molecular weight:	169.1 g/mol
CAS #:	1071-83-6
Structure:	

2. Non-Labeled Substrate: Androstenedione (ASDN)

CAS # :	63-05-8
Source:	Steraloids, Inc., Newport, RI (Catalog # A6030-100)
Batch # (expiration date):	L1712 (April 2016)
Purity:	99.8%

3. Radiolabeled Substrate: 1-β [^{3}H(N)]-Androst-4-ene-3,17-dione; ([^{3}H]ASDN)

Source:	Perkin Elmer, Boston, MA (Catalog #NET-926)
Batch # (expiration date):	619344 (January 10, 2012)
Radiochemical Purity (Supplier):	>97%
Specific activity:	26.3 Ci/mmol
Radiochemical Purity (In-lab determination):	Not determined

4. Positive Control: 4-hydroxyandrostenedione (4-OH ASDN)

CAS #	566-48-3
Source:	Sigma-Aldrich, St. Louis, MO (Catalog # F2552)
Batch # (expiration date):	081K2133 (March 2015)
Purity:	99.6%

5. Solvent (Vehicle Control): Dimethyl sulfoxide (DMSO) for 4-OH ASDN; Ethanol for ASDN and [^{3}H]ASDN; 0.1 M Sodium phosphate buffer for glyphosate

Sources:	Not reported
Batch #s (expiration date):	Not reported
Justification for choice of solvents	DMSO and ethanol are listed as vehicles acceptable for use in OCSPP 890.1200. Justification was provided for the use of DMSO as a solvent for 4-OH ASDN and for the use of 0.1 M sodium phosphate buffer as a solvent for glyphosate.
Concentration (% of total volume in assays)	≤1% DMSO; concentration of ethanol was not reported

6. Test Microsomes: Human recombinant aromatase (CYP19) microsomes

Source:	BD Gentest™, Woburn, MA (Catalog # 456260)
Lot # (expiration date):	19701 (July 2014)
Protein concentration:	3.7 mg/mL
Cytochrome C reductase activity:	540 nmol /mg protein/min
Aromatase activity:	5.7 pmol/pmol P450/min

B. METHODS

1. Assay Components and Preparations: A mixture of non-labeled and radiolabeled [^{3}H]ASDN was prepared such that the final concentration of ASDN in the assay was approximately 0.1 µM, and the amount of tritium added to each incubation tube was 0.1 µCi.

Glyphosate was formulated in the assay buffer (0.1 M sodium phosphate buffer, pH 7.4) based on its high water solubility, and relatively low organic solubility. The positive control, 4-OH ASDN, was formulated in DMSO such that the volume of DMSO used per assay was no more than 1% v/v of the total assay volume to minimize the potential for the solvent to inhibit the enzyme. DMSO was selected because it is listed as one of the solvents of choice detailed in the EPA guideline; it not as volatile as ethanol and so evaporation was less of a concern in the assay, and is more accurate to pipette because of its density and viscosity. ASDN and [^{3}H]ASDN were formulated in ethanol and the assay buffer; no maximum assay concentration for ethanol was reported.

A stock solution of the positive control substance, 4-OH ASDN, was formulated in DMSO. Fresh dilutions of the stock solution were prepared in the same solvent as the stock solution on the day of use. Dilutions were prepared such that the target concentrations of the positive control substance (0.1-10,000 nM; Table 4) were achieved by the addition of 20 µL of the dilution for a final assay volume of 2 mL.

Human recombinant microsomes were purchased from BD Gentest™, and stored at −80 ± 10ºC (storage interval not reported). Microsomes were thawed and portioned into individual vials based on the protein concentration of the batch (0.008 mg/mL microsomal protein per tube) and returned to the freezer for storage (storage interval not reported) to minimize freeze-thaw cycles to no more than one. The final concentration was approximately 0.004 mg/mL of microsomal protein/assay tube.

Other assay components sodium phosphate buffer, propylene glycol, and NADPH are reported in Table 1.

TABLE 1. Assay Components and Conditions[a]	
Assay Factor	**Values**
0.1M sodium phosphate buffer (pH 7.4)	
Microsomal Protein	0.004 mg/mL[b]
NADPH	0.3 mM
[^{3}H]ASDN	100 nM
Propylene Glycol	5%
Temperature	37 ± 2°C
Incubation Time	15 min

a Data were obtained from p. 18 of the study report.
b The concentration of microsomal protein was optimized for microsomes that produce approximately 540 pmol product/(min x mg protein) and 5.7 pmol product/pmol P450/min.

2. Suitability Assessments: The protein concentration was determined from the package information provided by the vendor; protein concentration was not verified on each day the aromatase assay was run.

Aromatase activity in each lot of human recombinant microsomes was determined to demonstrate the presence of sufficient activity for analysis of glyphosate. The aromatase activity was determined to be 0.584-0.771 nmol·mg-protein^{-1}·min^{-1}, which was greater than the minimum recommended aromatase activity of 0.1 nmol·mg-protein^{-1}·min^{-1}.

3. Aromatase Assay: Each assay run contained 4 tubes for the full enzyme activity and background activity controls, respectively, and a full concentration curve in duplicate for the positive control, and in triplicate for the test substance were established.

The amount of ^3H_2O in the aqueous fraction was quantified for each assay tube by LSC, and aromatase activity was reported in units of nmol·mg-protein^{-1}·min^{-1}.

4. Demonstration of Proficiency: Proficiency testing of the CYP19 aromatase assay was conducted in three independent runs on April 8, 16, and 20, 2010.by the test facility. The raw data from these three runs included evaluation of the positive control, 4-OH ASDN and the four recommended proficiency chemicals (econazole, fenarimol, nitrofen, and atrazine).

Positive Control

(1) Initial Demonstration of Laboratory Proficiency: Data from an initial demonstration of laboratory proficiency were not reported. The positive control data from the three acceptable assay runs generally met the following criteria:

- Mean aromatase activity in the absence of an inhibitor was at least 0.1 nmol/mg-protein/min.
- Mean background control activity was ≤ 15% of the full activity control.
- Coefficient of variation (CV) for replicates within each sample type and concentration of 4-OH ASDN was generally <15%.
- Performance criteria (Table 2) were met, and served as guidance in identifying runs that provided parameters in the preferred ranges.

(2) Demonstration of Proficiency of New Technician for Conducting Assay (when applicable): The demonstration of proficiency of a new technician was not indicated. The positive control data for slope, top and bottom percent from the three acceptable assay runs met the criteria as listed in section (i) of OCSPP 890.1200.

TABLE 2. Performance Criteria for the Positive Control[a]

Parameter	Lower Limit Criteria	Upper Limit Criteria	Actual Lower Limit	Actual Upper Limit
Slope	−1.2	−0.8	−1.00	−0.92
Top (%)	90	110	98.36	100.62
Bottom (%)	−5	+6	−0.06	0.76
Log IC_{50} (M)	−7.3	−7.0	−7.30	−7.28

a Data were obtained from pages 19 and 30 of the study report.

b. Proficiency Chemicals: Although the finalized data were not presented (including top and bottom of the curve, Hill slope, and log IC_{50}), the raw proficiency data that were provided (DEST.48671304) appear to support the expected designations of inhibitor or non-inhibitor for each of the proficiency chemicals, as well as the positive control.

TABLE 3. Proficiency Chemicals[a]

Compound	CAS#	Class	Concentrations
Econazole	24169-02-6	Inhibitor	10^{-3} to 10^{-10}
Fenarimol	60168-88-9	Inhibitor	10^{-3} to 10^{-10}
Nitrofen	1836-75-5	Inhibitor	10^{-3} to 10^{-10}
Atrazine	1912-24-9	Non-inhibitor	10^{-3} to 10^{-10}

a Raw data were included in Excel file 890.1200 Aromatase DEST.48671304

5. Determination of Aromatase Activity with Test Chemical(s): The response of aromatase activity to the presence of eight concentrations of glyphosate per run, in triplicate, was tested during three independent runs (Table 4). Solubility was assessed (presence of cloudiness or a precipitate). If insolubility was observed at the highest test concentration for the first run, then the highest test concentration would be adjusted for the second and third runs at the highest test concentration that appeared soluble using log or half-log concentrations. The lowest concentration tested was 10^{-10} M. The full enzymatic activity was obtained at the two lowest concentrations of the test chemical to define the top of the concentration-response curve.

TABLE 4. Test Chemical Study Design for each Test Run[a]			
Sample Type	**Repetitions (Tubes)**	**Description**	**Reference or Chemical (M)**
Full Activity Control	4	All test components[b] plus solvent vehicle	N/A
Bkgd Activity Control	4	Same as above without NADPH	N/A
4-OH ASDN Conc 1	2	All test components plus 4-OH ASDN	1×10^{-5}
4-OH ASDN Conc 2	2	All test components plus 4-OH ASDN	1×10^{-6}
4-OH ASDN Conc 3	2	All test components plus 4-OH ASDN	$1\times10^{-6.5}$
4-OH ASDN Conc 4	2	All test components plus 4-OH ASDN	1×10^{-7}
4-OH ASDN Conc 5	2	All test components plus 4-OH ASDN	$1\times10^{-7.5}$
4-OH ASDN Conc 6	2	All test components plus 4-OH ASDN	1×10^{-8}
4-OH ASDN Conc 7	2	All test components plus 4-OH ASDN	1×10^{-9}
4-OH ASDN Conc 8	2	All test components plus 4-OH ASDN	1×10^{-10}
Glyphosate Conc 1[c]	3	All test components plus Glyphosate	1×10^{-3}
Glyphosate Conc 2[c]	3	All test components plus Glyphosate	1×10^{-4}
Glyphosate Conc 3[c]	3	All test components plus Glyphosate	1×10^{-5}
Glyphosate Conc 4[c]	3	All test components plus Glyphosate	1×10^{-6}
Glyphosate Conc 5[c]	3	All test components plus Glyphosate	1×10^{-7}
Glyphosate Conc 6[c]	3	All test components plus Glyphosate	1×10^{-8}
Glyphosate Conc 7[c]	3	All test components plus Glyphosate	1×10^{-9}
Glyphosate Conc 8[c]	3	All test components plus Glyphosate	1×10^{-10}

a Data were obtained from page 20 of the study report.
b The complete assay contained buffer, propylene glycol, microsomal protein, [^{3}H]ASDN, and NADPH.
c Test chemical.

C. DATA ANALYSIS

1. Raw Data: Raw data were converted to aromatase activity (nmol·mg−protein^{-1}·min^{-1}) and percent control for the positive control and test chemical. The following raw data and calculated endpoints for each run were included in the report (Table 5).

TABLE 5. Raw and Calculated Data	
Raw/Calculated Data	**Included (X)**
DPM/mL for each portion of extracted aqueous incubation mixture	X
Average DPM/mL for each aqueous portion (after extraction)	X
Total DPM for each aqueous portion (after extraction)	X
The total DPM present in the assay tube at initiation	X
The percentage of substrate converted to product	X
Total DPM after extraction corrected for background	X
Aromatase activity expressed in nmol/mg protein/min	X
Average aromatase activity in the full activity control tubes	X
Percentage of control activity remaining in the presence of various inhibitor concentrations	X

DPM Disintegrations per minute

2. Statistical Methods: For data generated at CeeTox, basic statistical analysis was performed on the data, which included means of replicates, standard deviation of the mean, standard error of the mean, and coefficient of variation.

The response curve was fitted by weighted nonlinear regression analysis using a 4-parameter regression model (XLfit; IDBS; Version 5.2.0.0, Fit Model 208). For each run,

the individual percent of control values were plotted versus logarithm of the test chemical concentration. The fitted concentration response curve was superimposed on the plot, with individual plots prepared for each run. The average percent of control values versus logarithm of test chemical concentration for the individual runs for each test chemical (with different symbols for each run) were included on the same graph with their respective fitted response curves. In addition, the average percent of control values for each run versus the logarithm of test chemical concentration were plotted on a separate graph along with the average concentration response curve across runs were superimposed on the same plot.

3. Interpretation of Results: Interpretation of the assay results was based on the average of three runs, using the categories presented in Table 6.

TABLE 6. Interpretation of Results[a]

Criteria		Interpretation
Data fit 4-parameter nonlinear regression model	Average curve across runs crossed 50%[a]	Inhibitor
	Average lowest portion of curves across runs is between 50% and 75% activity[b]	Equivocal
	Average lowest portion of curves across runs is greater than 75% activity[b]	Non-inhibitor
Data do not fit model	---	

a Data obtained from Table 9, p. 23 of the study report.
b Ordinarily, an inhibition curve will fall from 90% to 10% over 2 log units with a slope near −1. Unusually steep curves may indicate protein denaturing or solubility issues. If the slope of the curve is steeper than −2.0, the result is classified as equivocal.
c If the test compound was not soluble above 10^{-6} M and the inhibition curve does not cross 50%, the chemical is typically determined to be untestable in the aromatase assay.

II. RESULTS

A. CONTROL ACTIVITY: Aromatase activity in the full activity controls ranged from 0.584-0.771 nmol·mg-protein^{-1}·min^{-1} for the 3 test runs, with a mean and standard deviation of 0.676 ± 0.072 nmol·mg-protein^{-1}·min^{-1}. Activity in the background controls ranged 0.23 to 0.38% and averaged 0.30% of the full control activity. The response of the full activity controls and background controls were acceptable for each run.

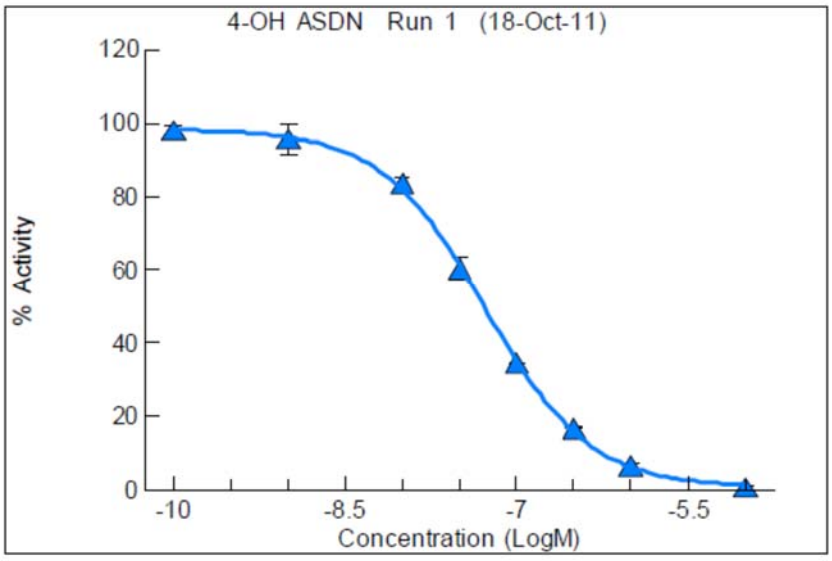
B. POSITIVE CONTROL: For the positive control substance (4-OH ASDN), aromatase activity averaged 0.668 ± 0.069 nmol·mg-protein^{-1}·min^{-1} at the lowest tested concentration 10^{-10} M and 0.005 ± 0.001 nmol·mg-protein^{-1}·min^{-1} at the highest tested concentration 10^{-5} M. The mean aromatase activity of the positive control (expressed as % full control activity) for each concentration tested across all 3 runs is presented in Table 7, along with the overall standard deviation, SEM, and %CV. An example of the inhibition response curve for the positive control from one run is shown in Figure 1.

TABLE 7. Effect of Glyphosate on Aromatase Activity (as percent of control) from Independent Runs[a]

Chemical	Concen. (Log M)	# Runs	Overall Mean[b]	Overall SD[b]	Overall SEM[b]	Overall % CV[b]
4-OH ASDN (positive control)	−5	3	0.67	0.04	0.02	5.4
	−6	3	5.95	0.45	0.26	7.5
	−6.5	3	15.73	1.07	0.62	6.8
	−7	3	34.02	0.48	0.27	1.4
	−7.5	3	61.47	1.17	0.68	1.9
	−8	3	82.56	1.01	0.58	1.2
	−9	3	98.14	2.63	1.52	2.7
	−10	3	98.77	1.54	0.89	1.6
Glyphosate	−3	3	109.30	5.53	3.19	5.1
	−4	3	109.73	7.16	4.14	6.5
	−5	3	106.72	1.67	0.96	1.6
	−6	3	102.75	2.45	1.41	2.4
	−7	3	104.48	1.05	0.61	1.0
	−8	3	100.67	1.50	0.87	1.5
	−9	3	102.22	0.59	0.34	0.6
	−10	3	99.67	1.86	1.07	1.9

a Data were obtained from Appendix 1, pp. 42-55 of the study report
b Calculated by the reviewers from data presented in this table.
SD Standard Deviation
SEM Standard error of the mean
CV Coefficient of Variance

FIGURE 1. Inhibition Response Curve for 4-OH ASDN From Run 1.



C. TEST SUBSTANCE: For glyphosate, aromatase activity averaged 0.673 ± 0.066 nmol·mg-protein^{-1}·min^{-1} at the lowest tested concentration, 10^{-10} M and 0.741 ± 0.100 nmol·mg-protein^{-1}·min^{-1} at the highest tested concentration, 10^{-3} M. The mean aromatase activity of glyphosate (expressed as % full control activity) for each concentration tested across all 3 runs is presented in Table 7 (presented above), along with the overall standard deviation, SEM, and % CV. Inhibition response curves for glyphosate from each run are

shown in Figure 2, and the average inhibition response curve across all runs is shown in Figure 3.

FIGURE 2. Inhibition Response Curves for Glyphosate From Each Test Run.

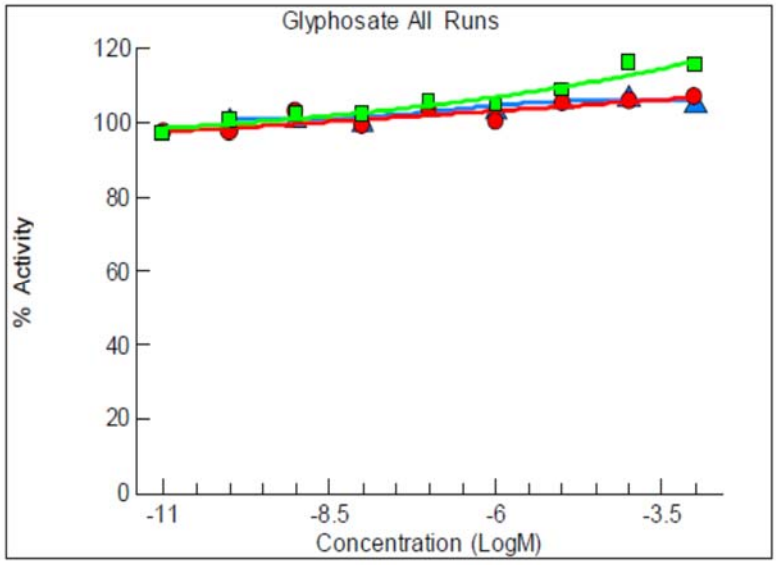
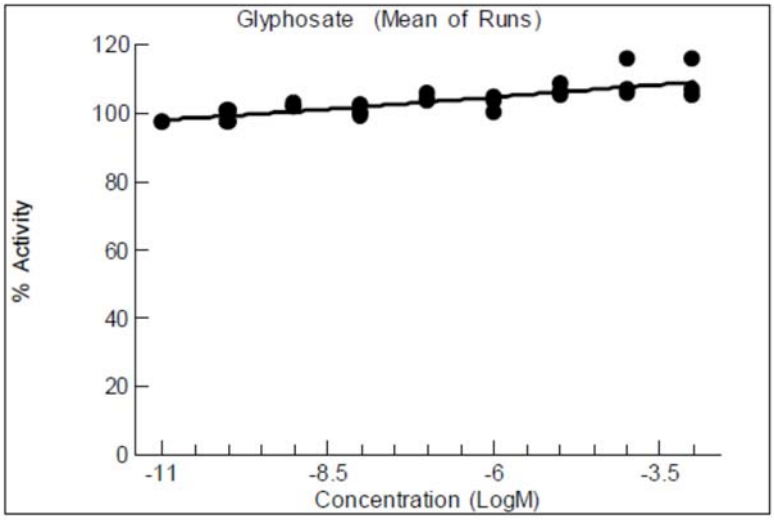


FIGURE 3. Mean Inhibition Response Curves for Glyphosate.



The effect of glyphosate on inhibition of aromatase activity is presented in Table 8. Log IC_{50} and Hill slope estimates were not determined for glyphosate because it never achieved >25% inhibition and could not be fitted by the nonlinear regression model. For 4-OH ASDN, the estimated log IC_{50} averaged −7.29 M and the Hill slope was −0.96 (Table 8). Confidence in these numbers is high due to the relatively small variation.

TABLE 8. Effect of Glyphosate on Aromatase Activity (as Percent of Control) From Independent Runs[a]						
Chemical	**Run 1**	**Run 2**	**Run 3**	**Mean[b]**	**SEM[b]**	**%CV[b]**
Log IC_{50} (M)						
Glyphosate	NA	NA	NA	NA	NA	NA
4-OH ASDN	−7.28	−7.30	−7.29	−7.29	0.01	0.14
Hill slope						
Glyphosate	NA	NA	NA	NA	NA	NA
4-OH ASDN	−0.96	−0.92	−1.00	−0.96	0.04	4.17

a Data were obtained from Table 13, page 30 of the study report
b Calculated by the reviewers from data presented in this table.
SD Standard Deviation
CV Coefficient of Variance
NA Not applicable

Based on the data from the average response curve and the criteria listed above in Table 8, the results support the conclusion that glyphosate is a non-inhibitor in the aromatase assay.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS CONCLUSIONS: Glyphosate at the highest soluble concentration of 10−3 M did not inhibit aromatase activity, and had a mean relative activity of 109% (n=3 runs) of vehicle control activity. Therefore, glyphosate was classified as a non-inhibitor of aromatase, as defined by EDSP guideline OCSPP 890.1200.

B. AGENCY COMMENTS: Results of proficiency testing for the aromatase assay were provided as raw data. Although the final calculation of parameters (including top and bottom of the curve, Hill slope, and log IC_{50}) were not provided, the raw proficiency data that were provided appear to support the expected designations of inhibitor or non-inhibitor for each of the proficiency chemicals, as well as the positive control.

Aromatase activity in the full activity controls was 0.676 ± 0.072 nmol·mg-protein^{-1}·min^{-1},and activity in the background controls ranged 0.23 to 0.38% and averaged 0.30% of the full control activity. The response of the full activity controls and background controls were acceptable for each run.

For the positive control substance (4-OH ASDN), aromatase results were within the recommended ranges for the top of the curve, bottom curve, Hill slope, log IC_{50}, and %CV for replicates of each concentration within runs. The estimated log IC_{50} for 4-OH ASDN averaged −7.29 M and the Hill slope was −0.96.

For glyphosate, average aromatase activity was ≥99.67% at the lowest and highest tested concentrations tested, 10^{-10} and 10^{-3} M, in each run. Since the lowest portion of the response curve across runs was greater than 75% activity at all concentrations, glyphosate is classified as a non-inhibitor of aromatase activity in this assay.

C. STUDY DEFICIENCIES: The following deficiencies were noted that are not considered to have had an adverse impact on the results, interpretation or conclusions of this study:

- The stability of glyphosate was not reported.

- For 4-OH ASDN, the CVs were >15% in separate instances for Runs 1 and 2 (15.9% for 10^{-6} M and 25.5% for 10^{-5} M).